Cell fate after DNA damage

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CHAPTER 7

SUMMARIZING DISCUSSION

SUMMARY

To maintain genome integrity, cells are equipped with a cell-intrinsic signaling network called the DNA damage response (DDR). This network recognizes DNA lesions and initiates various downstream pathways to coordinate a cell cycle arrest with the repair of the damaged DNA. If the levels of DNA damage are excessive, either due to the amount of inflicted DNA damage or due to insufficient DDR, the DDR can mediate clearance of affected cells through apoptosis or senescence. The cellular response to DNA damage is relevant for the maintenance of genome stability in normal tissues, as well as in cancer cells, of which treatment often involves DNA damaging therapeutics. However, not all damaged cells that are beyond repair are cleared and not every tumor from the same tumor type responds similarly to DNA damaging treatment. In this thesis, we aimed to identify factors that determine cell survival in situations of defective DNA repair, and uncover which factors determine sensitivity to cell cycle checkpoint inhibition or cisplatin treatment. Additionally, we investigated the effects of cell cycle checkpoint inhibitors on DNA repair.

Aberrations in cell cycle checkpoints or defects in DNA repair can lead to mitotic entry in the presence of DNA damage. To provide insight into how cells deal with DNA damage during mitosis, we performed a literature study in **Chapter 2**. Throughout the cell cycle, cells activate the DDR in response to DNA damage, although the extent of DDR signaling is different in each phase of the cell cycle. In the 1950s, it was recognized that when cells are damaged during mitosis, they continue cell cycle progression without repairing their DNA. This contrasts with interphase cells, which arrest cell cycle progression to allow DNA repair. Recent findings revealed that the DDR is partially turned off during mitosis. In response to irradiation-induced DNA double strand breaks (DSBs), ATM is activated, the MRN complex is still recruited to sites of DNA damage during mitosis and H2AX is phosphorylated. However, downstream DDR components, including 53BP1, are not recruited to irradiation-induced foci, nor are histones ubiquitinated. Thus, mitotic cells appear to mark their DNA damage for repair after mitotic exit.

Cyclin-dependent kinase 1 (CDK1) is one of the mitotic kinases that inactivates key DNA damage checkpoint proteins when cells enter mitosis. In **Chapter 3,** we investigated if forced CDK1 activation during interphase affects the repair of DNA double strand breaks. We elevated the activity of CDK1 using the chemical WEE1 inhibitor MK-1775. In physiological situations, WEE1 phosphorylates CDK1 to keep CDK1 inactive, and chemical WEE1 inhibition thus elevates CDK1 activity. We validated in non-transformed cells that WEE1 inhibition does not induce cytotoxicity in normal cells. Instead, we found that WEE1 inhibition potentiates the sensitivity to radiotherapy and accelerated mitotic entry in p53-defective cancer cells, but not in p53-proficient cancer cells. We further observed an impaired DNA damage response after forced CDK1 activation. Specifically, WEE1 inhibition reduced the numbers of 53BP1 foci at sites of DNA damage while it increased γH2AX levels, indicative of defective DNA repair. Indeed, when CDK1 levels were increased in interphase cells, homologous recombination (HR) repair was compromised, as confirmed by *in vivo* endonuclease assays. We found that this defect in HR after WEE1 inhibition was accompanied by increased phosphorylation of the HR repair protein BRCA2, which

is one of the targets of CDK1. Combined, our findings indicate that forced activation of CDK1 in interphase cells interferes with normal DNA damage responses and impairs HR DNA repair. This mechanism can (at least partly) explain the observed effects of WEE1 inhibition on the cytotoxicity of chemo- and radiotherapy, and highlights WEE1 inhibition as powerful tool to modulate DNA damage responses in cancer cells.

To optimally facilitate patient selection for WEE1 inhibition and undercover potential resistance mechanisms, identification of genes that - beyond p53 inactivation - determine WEE1 inhibitor sensitivity is necessary. Therefore, in **Chapter 4** we performed an unbiased functional genetic screen in a *TP53*-mutant background to identify mutations that confer resistance to the WEE1 inhibitor MK-1775. Despite the well-described function of WEE1 in the G2/M transition, insertion site mapping of cells that survived long-term WEE1 inhibition revealed enrichment of G1/S regulatory genes. In line with this observation, we observed that WEE1 inhibition during S phase was more cytotoxic, when compared to treatment of non-S phase cells. Moreover, cells specifically accumulated H2AX phosphorylation during S phase progression in response to WEE1 inhibition. Using a combination of live cell microscopy with FUCCI cell cycle reporters, we showed that WEE1 inhibition abrogates G2 duration, resulting in cytokinesis failure and apoptosis. Interestingly, stable depletion of G1/S regulatory genes SKP2, CUL1 and CDK2 reduced WEE1 inhibition-induced γH2AX levels, rescued G2 phase duration and caused resistance to WEE1 inhibition in breast and ovarian cancer cell lines. Remarkably, depletion of SKP2, CUL1 and CDK2 did not rescue the WEE1 inhibition-induced cytokinesis defect. In conclusion, we have identified components of the G1/S transition as determinants of WEE1 inhibitor sensitivity, indicating that interrogating G1/S control, beyond p53 status, could serve as a selection criterion for WEE1 inhibitor eligible patients.

Triple-negative breast cancer (TNBC) is an incompletely understood tumor type with a worse overall prognosis when compared to other breast cancers. Platinum-based chemotherapeutics, such as cisplatin, previously appeared to be effective when tested in *in vitro* TNBC models. These observations were explained by TNBC cancers frequently harboring DNA repair defects. However, we observed that TNBCs displayed great diversity in cisplatin-sensitivity, which could not solely be explained by cancer-associated defects in DNA repair. To better understand and aid in developing tools to predict cisplatin sensitivity, we applied a systems-level analysis of cisplatin responses in TNBC cells. To this end, in **Chapter 5**, we used quantitative time-resolved signaling data and phenotypic responses in combination with mathematical modeling. The mathematical model we built was based on partial-least-squares (PLS) regression analysis, and could accurately predict cisplatin-induced cell death, both in an experimental panel, as well as a validation set of TNBC cell lines. Interestingly, dynamics of signaling, rather than absolute activation status, were shown to be critically important to distinguish cisplatin-sensitive from resistant cell lines. Specifically, the dynamics of G3BP2 and MK2 activation were shown to determine cisplatin sensitivity, and inactivation of these genes conferred cisplatin sensitivity in resistant cell line models. Combined, we provided a time-resolved map of cisplatin-induced signaling, which uncovered new determinants of chemo-sensitivity and offers starting points to optimize treatment efficacy.

Functional repair of double-stranded breaks via HR is vital to maintain genomic integrity in living cells and is therefore essential to cellular viability. Surprisingly, however, loss of HR-related genes such as BRCA2 is tolerated in cancer cells and stimulates the development of breast and ovarian cancer. This apparent contradiction is called the 'BRCA paradox'. In **Chapter 6,** we performed a genome-wide loss-of-function genetic screen to identify genes that rescue the cell death induced by *BRCA2* inactivation. Mapping of the insertion sites showed that inactivation of the TNF receptor (TNFR1) or its downstream effector SAM68 rescued cell death induced by BRCA2 inactivation in KBM-7 cells. As a mechanistic link between the TNF-signaling pathway and BRCA2 function, we showed that BRCA2 inactivation induced TNFα production, and increased downstream signaling of the TNF receptor as measured by phospho-JNK and enhanced TNFα sensitivity. This increase in TNFα sensitivity could be rescued by inactivation of TNFR1 or SAM68, and was not restricted to BRCA2 inactivation, as depletion of BRCA1 or FANCD2, or hydroxyurea treatment also sensitized cells to TNFα. Finally, quantitative mass-spectrometry revealed that upon BRCA2 depletion, cells upregulate NF-kB activation, likely as a compensatory mechanism to counter increased TNFα signaling. In conclusion, our data reveal a novel mechanism by which autocrine TNFα signaling, induced by loss of BRCA2, limits tumor cell viability.

DISCUSSION AND FUTURE PERSPECTIVES

S phase genes determine WEE1 inhibitor sensitivity

WEE1 is a dual specificity kinase that phosphorylates, and thereby inhibits both CDK1 and CDK2 at tyrosine 15 (Y15)[1]. Together with their cognate cyclins, CDKs form cyclin/CDK complexes, which are the key drivers of the cell cycle. In conjunction with Cyclin B, CDK1 is involved in the progression from G2 phase to mitosis, while CDK2 complexed to either Cyclin E or Cyclin A is involved in the transition from G1 to S phase and the progression through S phase[2]. When CDKs are inhibited, for instance through WEE1-mediated Y15 phosphorylation, (unscheduled) cell cycle progression is prevented. Conversely, activation of CDK1/2 kinases requires Y15 to be dephosphorylated by one of the CDC25 phosphatases[3]. Combined, WEE1 is an important cell cycle checkpoint kinase that prevents premature S phase and mitotic entry.

Chemical inhibitors of WEE1 are currently being tested clinically as targeted anti-cancer drugs. For long, the consensus view was that WEE1 inhibitor-induced cytotoxicity is mediated by G2/M checkpoint inactivation[4,5]. Specifically, loss of the G2/M checkpoint would force premature mitotic entry, leading to mitotic catastrophe[6]. In line with this model, WEE1 inhibition is preferentially effective in *TP53*-mutant cancer cell lines that lack a functional G1/S checkpoint and depend strongly on their G2/M checkpoint control for genomic stability[7]. However, in our study we uncovered determinants of WEE1 inhibitor sensitivity which do not directly control G2/M checkpoint behavior[8]. The lack of G2/M gene mutations was not likely due to these genes being essential *per se*. For example, CDC25C is a clear G2/M regulator and appears not to be an essential gene and was not identified in our screen[9]. Rather, we identified genes that control the G1/S phase transition and observed that WEE1 inhibition is

preferentially cytotoxic in S phase cells. Indeed, knockdown of S phase genes or chemical inhibition of CDK2 rescued the γH2AX induction and the cytotoxicity induced by WEE1 inhibition. In line with our observations, down-regulation of CDK2, but not CDK1, was previously shown to rescue the accumulation of γH2AX in WEE1-depleted U2OS osteosarcoma cells[10]. In addition, cell lines with constitutive CDK2 activity (though expression of the CDK2AF allele) showed accumulation of irreparable DNA damage during S phase, which could only be prevented by reducing the levels of Cyclin A/CDK2[11].

The importance of CDK2 activity in cancer cells was illustrated by studies that investigated the CDK2 activation status or its transcriptional signature across tumor samples. Specifically, high CDK2 activity in frozen biopsy samples taken before neoadjuvant chemotherapy was associated with high pathological complete response rates to paclitaxel[12,13]. Similarly, a 97-gene transcriptional signature that proxies CDK2 activity predicted that cancers with low CDK2 activity profile had a poor outcome, while particularly in colon cancer, high CDK2 activity was associated with improved outcome[14]. These data imply that in the context of chemotherapeutic treatment, low CDK2 activity is protective for cancer cells, and conversely, that high CDK2 activity improves outcome. These data are in good agreement with our results on WEE1 inhibition.

Around the time that we performed our functional genetic screen, a genome-scale pooled RNA interference screen was reported using the same WEE1 inhibitor, MK-1775, in pancreatic cancer models[15]. In agreement with our data, cytotoxicity of MK-1775 was shown to be suppressed by knockdown of S phase related genes. Specifically, suppression of CDK2 and Cyclin A2 was identified to increase proliferation after WEE1 inhibition. Thus, although WEE1 is known to be required for proper G2/M checkpoint functioning, our results and those of others indicate that the cytotoxicity upon WEE1 inhibition is mediated primarily in S phase by regulating the CDK2 signaling axis. Importantly, CDK2 activity appears to be a more generic determinant of survival in the context of regular chemotherapy.
Surprisingly, when baseline expression levels of 200 total or phosphorylated proteins were correlated with MK-1775 sensitivity, high levels of AXL, an activator of the AKT/mTOR pathway, appeared to correlate with primary resistance to MK-1775 in small cell lung cancer (SCLC) models[16]. Although MK-1775 resistance was shown to be rescued through the addition of AXL or mTOR inhibitors, they did not test different WEE1 inhibitors or WEE1 siRNAs to exclude MK-1775 off target effects. A recent report in which the specificity of MK-1775 was investigated confirmed the presence of off target effects, including PLK1 and mTOR activators[17]. Unfortunately, the correlation of CDK2 or phospho-CDK2 levels with MK-1775 resistance could not be assessed, as the full dataset of this specific report was not disclosed. Combined, these results indicate that WEE1 inhibitor sensitivity is controlled by S phase regulators, but that additional, context-dependent, determinants likely play a role.

Checkpoint kinases are interesting therapeutic targets when applied based on careful analysis of the tumor to support patient selection

Immediately after DNA break detection, a cell cycle arrest is installed to provide cells the time to repair DNA breaks, and to prevent the transmission of damaged chromosomes to daughter cells. DNA

damage can induce an arrest at three distinct points during the cell cycle. The G1/S checkpoint arrests cells prior to entering S phase, the intra-S phase checkpoint controls ongoing DNA replication, and the G2/M checkpoint prevents entry into mitosis in case of unrepaired DNA lesions. Although these checkpoints act at distinct phases in the cell cycle, their molecular wiring shows significant overlap. In response to DNA DSBs, the common upstream regulator ATM becomes activated and phosphorylates and thereby activates the CHK2 kinase. In S/G2, ATR is activated in response to ssDNA stretches and activates CHK1. Consecutively, both CHK2 and CHK1 inhibit the CDC25 phosphatases and thereby inactivate CDKs. In parallel, activation of ATM, ATR, CHK2, and CHK1 all lead to activation of p53, which mediates a cell cycle arrest through transactivation of CDK inhibitors, notably p21.

As cells are under constant assault of DNA damage either through endogenous (such as replication errors) or exogenous sources (including sunlight), efficient DNA repair is vital. Not only do cells rely on a functional DNA repair machinery, also sufficient time to repair the damage is essential to prevent cells from progressing through the cell cycle in the presence of DNA damage. Therefore, checkpoint inhibitors are cytotoxic as single agents[4]. Most likely, fast replicating cancer cells produce high levels of replication stress-induced DNA damage, and therefore depend on functional cell cycle checkpoints for cell survival. In addition, checkpoint inhibitors were shown to potentiate genotoxic anti-cancer therapies including irradiation and chemotherapy[18,19]. The effects of 'sloppy' versus efficient checkpoint function were illustrated in Chapter 5, where we identified differential regulation of S/G2 cell cycle progression in cisplatin-sensitive versus resistant triple-negative breast cancer (TNBC) cell lines in response to cisplatin treatment. Whereas cisplatin-resistant TNBC cell lines arrested in response to cisplatin and only continued cycling when levels of DNA damage were reduced, cisplatin-sensitive cell lines maintained arrested or continued cycling in the presence of high levels of DNA damage. Notably, depletion of the cell cycle checkpoint component MK2 in a cisplatin-resistant cell line caused increased sensitivity to cisplatin. Interestingly, WEE1 inhibition was recently also found to overcome cisplatin resistance in TNBC cell lines and xenografts[20]. These findings illustrate that inactivation of cell cycle checkpoint components on which cells rely for survival in conditions of DNA damage can indeed be used to induce sensitivity of DNA damaging agents. Also, multiple other studies using a range of tumor models have confirmed an increased sensitivity to DNA damaging agents when key checkpoint players have been inhibited or depleted[21,22,23].

Chemical inhibitors have been developed for several cell cycle checkpoint kinases, including CHK1, CHK2, ATR, ATM as well as WEE1, and a number of such inhibitors are currently being tested in clinical trials. In case of the WEE1 inhibitor MK-1775, patients with *TP53*-mutated ovarian cancer, refractory or resistant (< 3 months) to first-line platinum-based therapy were included in a phase II study, in which MK-1775 was combined with carboplatin[24]. Interestingly, the combination treatment of MK-1775 with carboplatin demonstrated manageable toxicity and an overall response rate of 43% (95% confidence interval (CI), 22% to 66%); including one patient (5%) with a prolonged complete response. Although these results are very promising, clearly, *TP53* mutation status alone is not sufficient to select those patients that will benefit from WEE1 inhibition treatment.

Our results indicated that WEE1 inhibitor-insensitive tumor cells frequently undergo failed cytokinesis in response to WEE1 inhibition. Next to regulating proper S and G2/M checkpoint functioning, WEE1 appears to be required for accurate mitotic exit, in a CDK1-dependent fashion. CDK1 phosphorylates – and thereby inhibits – multiple cytokinesis components including MKIp2, ECT2 and Separase[25,26]. So, it is very likely that sustained CDK1 activity induced by WEE1 inhibition may underlie defective cytokinesis. In line with this observation, cells with constitutively active CDK1 (CDK1AF) enter and exit mitosis-like states without carrying out cytokinesis or karyokinesis[27]. Indeed, constitutively active CDK1 inhibits sister chromatid separation which can solely be prevented by interrupting the phosphorylation of Separase[26]. In addition, CDK1-dependent phosphorylation of SLX4 controls MUS81-SLX4 complex formation[28]. This complex promotes targeted resolution of persistent replication intermediates during mitosis, thereby safeguarding chromosome segregation. Thus, if cells survive the cytotoxic effects of WEE1 inhibition due to inactivated S phase regulators, WEE1 inhibition entails the risk of inducing cytokinesis failure and thereby increasing tumor aggressiveness[29]. For that reason, cancers with unusually low expression or activity of S phase regulators should be excluded from treatment with WEE1 inhibitors. Conversely, extraordinary high levels of CDK2 activity in cancer cells, for instance due to amplification of *CCNE1*, encoding the Cyclin E protein, may highlight those tumors that respond favorably to WEE1 inhibition. In line with this notion, patients with *CCNE1* amplified cancers were enriched in the group of responders to WEE1 inhibitor treatment[24], and a recent clinical trial studies WEE1 inhibitor treatment selectively in patients with *CCNE1* amplifications[30].

Dynamics versus steady-state levels of signaling molecules

Biomarkers that predict therapy response are typically measured in untreated tumor samples at their steady-state level. Some of these molecular markers are used to select cancers for targeted therapies, and have shown robust clinical value, as for instance with HER2 status for trastuzumab treatment. Surprisingly, in Chapter 5, we found that signaling dynamics, rather than steady-state levels, were important to predict cellular responses to cisplatin. We measured signaling flux at various time-points after initiation of cisplatin treatment, and found that dynamics were critically important in distinguishing cisplatin-sensitive from resistant cell lines. Of note, we were able to predict cisplatin response in a validation panel of TNBC cell lines by measuring the signaling flux of only five signals. Therefore, in an ideal scenario, samples before and shortly after start of treatment should be analyzed to timely predict responses to cisplatin treatment.

To implement signaling dynamics as predictive biomarker for treatment response, tumor material should be collected before and shortly after treatment. Repeat biopsies can be taken after treatment, and analyses of such samples has been shown to predict or improve treatment outcome[31,32]. Alternatively, tumor material can be cultured and treated *ex vivo*. Interestingly, using *ex vivo* cultures, multiple time-points and treatment options can be included. To date, several strategies have been applied to generate primary cultures from individual tumors, of which organotypic tumor slice cultures and patient-derived tumor organoids are two of them. Recently, the importance of measuring treatment

responses *in vitro* tumor models over sequencing the mutation status of genes to aid cancer drug-treatment decisions was again highlighted. Whole-exome sequencing (WES) information of tumor-normal pairs from 501 patients revealed that only around 10% had gene alterations in targetable cancer drivers[33]. Using organoids, a 3D stem cell-based cell-culture system, high-throughput drug screening was performed which revealed effective drugs and drug combinations that limited cancer-cell growth and were not identified with WES. The major disadvantage of organoids is that the expansion of tumor cells takes months of culturing. Using organotypic tumor slice cultures, tumor slices can directly be treated without expansion, and treatment-induced changes in signal dynamics can be measured with immunohistochemistry, immunofluorescence or western blotting[34,35,36]. Using these *in vitro* cancer models, dynamical biomarkers can potentially be used to guide precision medicine next to mutation status, beyond steady-state biomarkers.

Rescue of BRCA2 deficiency-induced cell death by reversing apoptotic signaling

BRCA1 and BRCA2 are two key players in homologous recombination (HR)-mediated DNA repair. While BRCA1 functions upstream in HR, where it controls DNA-end resection at sites of DNA double-strand breaks, BRCA2 functions downstream, where it controls the loading of the RAD51 recombinase. Without functional HR, DNA breaks accumulate, leading to cell cycle arrest and p53-induced apoptosis, underscoring the essential role of functional HR to maintain genome integrity and cellular viability. Strikingly, while loss of BRCA1/2 is not tolerated in normal cells, tumors with homozygous mutations in *BRCA1/2* are viable. An intriguing question is which additional (epi)genetic events allow tumor cells to survive BRCA1/2 loss. In general, *BRCA1* or *BRCA2* mutant cancers almost invariably have inactivated *TP53*. However, *Tp53* inactivation only partially rescued embryonic lethality and cellular viability of *Brca1/2* mutant cells, indicating that additional mechanisms are likely to play a role in the survival of these cells. In case of BRCA1, loss of 53BP1, RIF1, REV7 or HELB was shown to rescue BRCA1 deficiency[37,38,39,40,41]. Interestingly, all these mutations counteract reduced cell viability after BRCA1 loss by restoring HR. Mechanistically, 53BP1, RIF1 and REV7 act as barriers to DNA-end resection, and are normally inhibited by BRCA1 in S and G2 phase. Upon loss of BRCA1, CtIP-dependent end resection is impaired, but can be re-established if one of the barriers to end resection is lost. For BRCA2-deficient cancer cells, it remains less clear which gene mutations rescue cellular viability. Recently, inactivation of *PAXIP1* (encoding PTIP), was shown to rescue cell death induced by BRCA2 loss[42]. Instead of rescuing HR, loss of PTIP protects BRCA1/2-deficient cells from DNA damage by inhibiting MRE11 recruitment to stalled replication forks. Most likely, HR cannot be restored in BRCA2-deficient cells because BRCA2 functions further downstream in the HR pathway and is therefore more essential than BRCA1. RAD51 itself functions downstream of BRCA2 and is even more essential, as also underscored by the fact that inactivating RAD51 mutations have thus far not been observed in cancers. RAD51 paralogs, including RAD51C, facilitate RAD51 and are not essentially required for viability. Recently, RAD51C mutations have been identified in cancer[43].

In our unbiased genome-wide genetic screen with BRCA2-deficient cells (Chapter 6), we also identified PTIP. Yet, we also identified multiple TNF receptor related genes, which had more insertions and these hits were therefore more significant. We observed increased downstream signaling of the TNF receptor after BRCA2 loss, as well as increased sensitivity to TNFα. In other words, our results indicate that the TNFα signaling axis plays an important role in response to BCRA2 loss. Interestingly, TNF receptor signaling has been reported to promote apoptosis as well as cellular survival in a context dependent manner[44]. In case of DNA damage induced by DNA damaging agents or after loss of DNA repair genes, there is a delicate balance between survival and cell death. In case of sufficient DNA repair capacity, cellular survival is initiated, while accumulation of unrepaired DNA damage triggers apoptosis[45]. Quantitative mass-spectrometry showed an initial upregulation of proteins that activate NF-κB upon BRCA2 depletion. Yet, prolonged BRCA2 depletion promotes caspase-dependent apoptosis, which is mediated through ASK1/JNK signaling. We observed that caspase inhibition increased cellular viability, and likewise, that JNK inhibition reduced TNFα sensitivity of BRCA2 deficient cells. Of note, the rescue of cellular survival with the caspase inhibitor was only partial. Possibly, the residual amount of cell death induced by BRCA2 inactivation is mediated by caspase-independent apoptosis. Mitochondrial outer membrane permeabilization (MOMP) has recently been shown to trigger TNF-dependent, but caspase-independent cell death[46]. In this scenario, MOMP stimulates NF-kB activity, which initiates necroptosis. These data highlight in addition to triggering apoptosis, TNF signaling can induce necroptosis. In addition, these data show that NF-kB activity can also be linked to TNF-dependent initiation of cell death.

In good agreement with our data, BRCA2-deficient cancer cells were recently demonstrated to display increased sensitivity towards death-receptor mediated apoptosis. Very similar to what we observed in response to TNF receptor activation, in this report, activation of the TRAIL receptor strongly induced apoptosis in cells harboring *BRCA2* mutations[47]. Combined with our data, this suggests that BRCA2-mutant tumors may be selectively sensitive to TNF receptor 1 or TRAIL receptor-mediated apoptosis, which could facilitate novel therapeutic approaches for patients with BRCA2-deficient tumors.

CONCLUDING REMARKS

The way in which tumor cells respond to DNA damage varies between different tumor cells, as are the factors that underpin these cellular fate decisions. For instance, while some tumors are unable to repair the inflicted damage due to mutations in DNA repair genes like BRCA1/2, other tumors do not engage in a proper cell cycle arrest due to a failure in checkpoint activation. In addition, the DDR is not functioning as one linear signaling pathway, but rather employs several pathways that display crosstalk and feedback which results in many factors that determine cell fate. Finally, there are different types of DNA lesions induced by intrinsic or extrinsic factors which each causes a different cellular response and requires a specific type of repair. Taken together, the multitude of factors that determine cell fate after DNA damage harbors many opportunities for targeted anti-cancer therapies. Yet, the immense signaling

complexity also creates difficulties for predicting therapeutic responses, as these different factors can also be hijacked by tumor cells as 'escape mechanisms' to survive treatment-induced DNA lesions.

Successful implementation of targeted anti-cancer therapies requires proper patient selection. This is specifically important, because sub-optimal treatment (i.e. of tumors that are not very sensitive to a targeted agent) can cause the emergence of resistant tumor cell populations that show increasingly aggressive behavior and which are more difficult to treat. To implement efficient patient selection, biomarkers are needed for each molecularly targeted or chemotherapeutic agent. This holds true for drugs that direct or indirectly cause DNA damage, but is similarly important for other anticancer drugs. Most targeted agents that are used in the clinic are already linked to a particular biomarker. For instance, the application of trastuzumab is linked to HER2-receptor status, and the BRCA1/2 mutation status is a biomarker for patient selection for treatment with the PARP inhibitor olaparib. As illustrated in this thesis, genome-wide genetic screens constitute a perfect tool to discover potent biomarkers and resistance mechanisms. To complement data of haploid genetic screens, it will be very interesting to perform additional genetic screens that take into account the epigenetic and environmental changes, which have been shown to be relevant in tumor cell biology. Indeed, tumor development and responses of cancers to drugs is greatly influenced by interactions between tumor cells and the various different cell types that constitute the microenvironment[48]. Additionally, beyond genetic mutations that completely shut off genes, epigenetic changes have much more subtle effects on gene expression levels, and were shown to play a role in treatment response[49].

Whenever the vulnerability of a tumor is identified, a targeted agent – if present – can be selected to treat a selected patient. However, to identify a specific vulnerability of a tumor, tumor material should be collected and the mutation status and mRNA expression levels of predictive markers should be addressed. In addition, for every targeted therapy the mechanisms of resistance should ideally be known and predictive markers are desired. Since this knowledge is not present for all targeted agents that are currently in clinical trials, in depth investigation at the cell biological level is warranted to acquire an improved picture of the molecular mode of drug action, and the cellular responses they induce.

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